STUDIES ON THE NITROGEN REQUIREMENT AND METABOLISM OF SOME MEDICINAL PLANTS

Dissertation Submitted for the Degree of

Master of Philosophy

IN

BOTANY

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DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH

1986

Acknowledgement

I am deeply indebted and express my deep sense of gratitude to Dr. Arif Inam, Lecturer, Department of Botany, Aligarh Muslim University, Aligarh, and to Dr. S.H. Afaq, Reader, Department of Ilmul-Advia, A.K. Tibbiya College, Aligarh Muslim University, Aligarh, for their guidance and valuable suggestions at every step during the preparation of this manuscript. Their constant and affectionate supervision has always been a source of inspiration to me.

I am also grateful to Prof. M.M.R.K. Afridi for continued interest and encouragement at every step of my work and to the Chairman, Department of Botany, Aligarh Muslim University, Aligarh, for providing necessary research facilities.

I express my sincere thanks for the help and cooperation extended to me by my teachers, Dr. Samiullah and Dr. Aqil Ahmad and by my colleagues, Dr. Feroz Mohammad, Dr. Masood Akhtar, Dr. Shamim, A. Ansari, Dr. M.M.A.Khan, Mrs. Najma Pervez, Mrs. Akhtar Inam, Mr. Moinuddin, Mr.Mujahid A. Khan, Mr. Nafees A. Khan, Mr. Faizan A.Khan, Mr. Shahid Umar, Mr. S.R.M. Ata, Mr. Ikramul Haque, Miss Nasreen Fatima and Miss. S. Shama Muzzaffar.

I heartily thank my parents, sisters and brothers for their assistance and kind encouragement when it was most needed.

I am also thankful to Mr. Ziaur Rehman who willingly and deligently typed the manuscript.

And, in the last, but not the least, I am grateful to God Almightly without whose Grace this work would not have been completed.

Atiya Khatoon Zaidi

C O N T E N T S

CHAPTER - 1

INTRODUCTION

CHAPTER - I

INTRODUCTION

The beginning of life on earth has remained a controversial topic. However, we can say with certainty that when man made his appearance on earth, plant kingdom was already there to greet him. Sooner or later, Mother nature provided man the idea of curative nature of herbs. The claim can perhaps never be denied that inspite of his open rebellion against nature, man can never deny that he shall remain a disciple of nature itself. Similarly, the fact can hardly be contradicted that any effort to alienate man from nature will only make his body system more and more prone to diseases. The co-existence of life with diseases, decay and death has naturally attracted the attention of man from the dawn of human civilization.

The detailed knowledge of medicinal plants must have been accumulated in the course of many centuries. The oldest records of medicinal plants are from Egypt, China and Sumero-Akkadian or the Mesopotamian region. The earliest Chinese records go back to 5,000 B.C. Among the medicinal systems of antiquity, the Egyptian system was the most advanced with more than 700 drugs compiled in the sixteenth century B.C. (Said,1982)

The history of Indian medicinal plants shows that Indo-Aryans were familiar with a good number of medicinal plants (Chopra et al. 1958). The vedic-Aryans were acquainted with about a hundred of medicinal plants. In Susruta, the properties and uses of some foreign drugs, which were imported in this country, were recorded. The works of Charka and Susruta appear to have been composed in pre-Buddist period. The rise of Budhism gave further impetus to the systematic cultivation and investigation of medicinal plants (Kirtikar and Basu, 1975).

The rise of Islam brought about a new era in the history of medicine. During this period, physicians and Hakims wrote a great deal on this aspect. They payed great attention to the study of indigenous drugs. There are several publications, in Arabic and Persian on indigenous drugs. Later, the Moghals not only brought with them their own Materia Medica and enriched this knowledge further, but also patronised the flourishing perfumery industry in India (Nadkarni,1982).

It was during the British period that the knowledge of indigenous medicinal plants was pursued more intensively on scientific lines and introduction of new medicinal plants from abroad was encouraged.

About 75 per cent of the medicinal plants mentioned in various pharmacopoeias of the world are grown in India and she

produces about 2,000 types of these plants. It is significant to note that pharmaceutical and perfumery industries have started establishing their own research and development units in many parts of the country and that quite a good number of medicinal plants, like belladonna, mentha and Pyrethrum, are cultivated on a large scale for their active constituents that are utilized indigenously as well as exported to earn precious foreign exchange.

The principal botanical drugs which have a good market in India as well as outside are aconite, aloe, belladonna, benzoin, cinchona, dioscorea, ephredra, ergot, ginseng, henbane, ipecac, isubgol, liquorice, opium, pyrethrum, rauvolfia, senna, stramonium, valeriana, vinca etc.

The first international congress on Medicinal Plant Research was held at the University of Munich (FRG). It was estimated that, out of 600,00 plant species existing on our planet, only five per cent have been investigated chemically and pharmacologically which highlights the vast scope for research to be done on medicinal plants.

The easy availability, low cost and miraculous action of herbal drugs make them ideally suited for the masses of developing countries like India. It is, therefore, highly desirable that the entire subject of drug procurement, produc-

tion and standardisation should be taken up with greater scientific care. This will be a safeguard against adulterated, spurious, soiled and substandard drugs. As a first step for successful economical cultivation of drugs of poorer quality and standard, it seems desirable to undertake large scale cultivation of at least some of the medicinal plants that are either getting extinct or whose supply is lagging behind their demand which leads to all sorts of malpractices, including inflated prices and/or adulteration. Unfortunately, not much research has been done on the requirements of fertilizers and other inputs of all but a few of these plants.

The need for much work on intensive lines has been felt at Aligarh, where a strong school of mineral nutrition of crop plants has developed during the last few decades. Since the mid 1970's, Afridi et al. (1977, 1978); Afaq (1978); Afaq et al. (1978, 1984); Wasiuddin (1979); Samiullah and Varshney (1981); Samiullah et al. (1982); Wasiuddin et al. (1982); Afridi et al. (1983); Varshney (1983); Khan (1984); Khan (1985) and Khan et al. (1985) have devoted full attention to the fertilizer requirements of a number of medicinal and aromatic plants, including Anethum, Cassia, Cichorium, Cymbopogon, Foeniculum, Plantago, Solanum and Datura.

Among the medicinal plants, Datura occupies an important position. It is poisonous and is distributed through-

-out the tropical and warm temperate region of the world. Ten species are found in India, of which Datura innoxia, Datura metel and Datura stramonium are medicinally important. The whole plant is toxic, narcotic, aphrodisiac and removes the pain of tumours and piles.

The young leaves are antispasmodic, anodynous and narcotic. The dried leaves and stem are smoked as an anti-spasmodic in asthma, whooping cough, bronchitis etc. The leaves are used in other treatments. They are mixed with wine or powdered rice and saffron and are applied externally for various pains and swellings. In Gold Coast the leaves are crushed and mixed with oil to be used as antidote against poisonous insect bites. In Guinea, the powdered leaves are applied to swellings, tumours and rheumatic pains. In our own country, fresh leaf juice is a popular remedy for pains, swellings, numps etc. The juice, boiled with oil is useful for skin diseases and running sores. It is also employed as a dressing for piles, fissures and other ailments of the rectum. The flowers are dried and roughly powdered with or without the leaves and rolled into cigarettes for the relief of asthma. The juice of the fruit is a useful dressing for the scalp to check dandruff and falling hair. The pests are used for decaying teeth, piles and parasitic skin diseases. The young fruits are said to be sedative and slightly into-xicating. The seeds are recommended in bites of mad dog and

purulent discharges from the ears. The roots is powdered and applied to the gums in order to relieve toothache (Dastur, 1962).

Some species of Datura like Datura stramonium are rich in hyoscyamine, while Datura innoxia and Datura metel are rich in scopolamine. Stramonium may also be used as a source of atropine on commercial scale (Anonymous, 1952). However, it has been noticed that the seeds of Datura obtained even from prestigeous suppliers are commonly a mixture of two or more species and, therefore, their efficacy in a particular ailment may not be guarantied (Afaq, unpublished).

In view of this and due to availability of meagre literature on the cultural practices of various species of Datura, it has been decided to undertake detailed studies on fertilizer requirements of only one species, namely Datura metel, by undertaking the following pot trials.

Experiment 1: Authentic seeds of Datura metel will be collected after rigorous identification of the species. These will be sown in autumn with various doses and sources of commercially available nitrogenous fertilizers. The soil used for this and subsequent pot experiments will be analysed before the application of the fertilizers. The aim of this experiment will be to work out the dose and source of nitrogen on the bases of growth, yield, NPK content , NRA, chlorophyll content and alkaloid percentage of the plant.

Experiment 2: The second pot culture experiment will be a repetition of experiment I with the difference that it will be conducted in the following summer. The aim of this study will be to observe the difference in growth characteristics, NPK concentrations, NRA, chlorophyll content and alkaloid percentage in Datura metel due to the change in the time of planting.

Experiment 3: In the following year, the optimum dose and suitable source selected after the above pot culture studies (Experiments 1 and 2) will be applied in split doses, (sub-optimal basal application at sowing and supplemental top-dressing at suitable stage of growth). The effect of GA_3, KI and pyridoxine soaking of seeds on nutrient uptake, yield attributes and the alkaloid content will also be investigated.

Experiment 4: Side by side with experiment 3, another trial will be conducted to study the effect of various doses of sub-optimal basal nitrogen supplemented with foliar application of a dilute aqueous solution of urea. The aim of this experiment will be to establish whether or not fertilizer economy is possible by using the technique, on the basis of the effect on various parameters studied in earlier trials.

The results will be analysed statistically according to the design of each experiment.

C H A P T E R - 2

REVIEW OF LITERATURE

C O N T E N T S

CHAPTER - 2

REVIEW OF LITERATURE

2.1 Historical - The application of farm yard manure to soil to boost crop productivity is probably as old as agriculture itself. Until about the middle of the last century, it was believed that the entire nutritional requirements of crop plants were provided by applying adequate quantities of organic fertilizer to the soil before sowing.

Woodward (1699) worked out that plants grew better in water containing dissolved solids. Home (1775) was of the opinion that plant nutrition depends on several factors. He established two methods for studying plant nutrition, namely "pot culture" and "plant analysis". de Saussure (1804) for the first time chemically analysed the soil as well as the plants grown in it and concluded that the composition of the plant varies with that of the soil. Sprengel in 1839, mentioned the importance of nutrient elements absorbed by the plant from the soil. Credit also goes to Boussingault for laying the foundation of modern agricultural field trials. Liebig, a German scientist is credited for humus theory according to which organic matter of the soil is the source of carbon that

plants absorbs. He considers that soil contributes soluble organic constituents. Sachs (1860) and Knop (1861) prepared solution of salts supplying the major essential mineral nutrient solutions containing salts of nitrogen, Phosphorus, Potassium, Calcium, magnessium, Sulphur and iron perfected the technique of "water culture", and established this new research technique in plant nutrition by growing them in nutrient solution (Bould, 1963, Epstein 1978).

2.2 Role of nitrogen, phosphorus and potassium (NPK)

Out of the list of elements required a large quantities (macronutrients), nitrogen, phosphorus and potassium have been the subject of innumerable field trials by farm scientists in various countries, particularly since the beginning of the present century as they are removed in very large quantities. Their metabolic roles in plants have received considerable attention of plant physiologists. The various ways in which these three macronutrient elements influence plant growth and reproduction are well known. They play varied roles in osmotic phenomena, as fundamental constituents of metabolic products and in several other ways. Their roles in plants are summarized below.

2.2.1 Nitrogen

Nitrogen is essential for plant growth as it is a constituent of all proteins and of protoplasm. It is generally taken up by the plants as ammonium or as nitrate ions, which is reduced and incorporated into organic compounds (Bandurski 1965). Nitrogen encourages the vegetative development of plants, imparting a healthy green colour to the leaves. Such leaves have larger surface available for photosynthesis. Nitrogen is an integral part of a large number of essential organic compounds, including aminoacids, coenzymes, porphyrins, purines, pyrimidines, nucleotides, nucleosides, chlorophyll, some vitamins, alkaloids and growth hormones (McKee 1962). An excess of nitrogen produces leathery dark green leaves and succulent growth, while its deficiency results in various disorder. e.g., retardation of shoot growth and root development, chlorosis etc. According to Steward and Durzan in 1965 it is "difficult to name a basic property of a cell in plants that does not at some point make its contact with their nitrogen metabolism".

2.2.2 Phosphorus

Phosphorus is not present in abundance in most soils. Wherever present, most of it is 'fixed' and thus

unavailable to plants. It is essential to supply fresh quantities of this nutrient to the soil each season for healthy growth of a crop. It is absorbed by plants as monovalent ($H_2PO_4^-$) or divalent (HPO_4^{--}) anions from soil. Much of the phosphate is converted into organic forms upon entry (Salisbury and Ross 1977).

Some of the important cell constituents, that have phosphorus as their integral part, include sugar esters and their derivatives, phospholipids, nucleosides, nucleotides, nucleic acids and nucleoproteins as well as coenzyme, including NAD, NADP, pyridoxal phosphate, TPP and ATP.

Phosphorus participates directly in the photochemical events of photosynthesis. Phosphate plays a key role in the energy transfer in respiration and other vital processes (Devlin, 1981).

Phosphorus, as orthophosphate, plays a fundamental role in a very large number of enzymatic reactions that depend on phosphorylation, possibly for this reason it is a constituent of cell nucleus and is essential for cell division and for the development of meristematic tissues. Phosphorus improves the quality of crop and also provides vigour and health. It encourages the formation of new cells, promotes root growth,

hastens flowering and ensures the formation of seed and grain. Its deficiency causes failure to make a quick start and growth remains stunted.

The importance of phosphorus for plants is thus well established. According to Hewitt (1963) it is involved in practically every synthetic reactions of the cell". It thus influence both the vegetative as well as the reproductive phase of plant life.

2.2.3 Potassium

Unlike nitrogen and phosphorus, potassium does not form a stable structural part of any molecule inside the plant cell. However, it is abundantly present in soluble form in the cytoplasm and in the vacuolar sap. It exists in the soil in non-exchangeable (fixed), exchangeable, and soluble form. It has been reported that potassium activates the enzymes that synthesize certain peptide bonds and enhances the incorporation of amino acids into protein (Webster, 1953, 1956). Potassium plays an important role in activating numerous enzyme systems (Nason and McElroy, 1963). It enhances the plants ability to resist diseases, insect attacks, cold and other adverse conditions (Evans and Sorger, 1966).

Potassium also plays an important role in stomatal opening (Humble and Hsiao, 1969). It influences the hydrature of the protoplasm. The uptake of some other elements by roots and their subsequent distribution in the plant is also related to the potassium status of the soils and plants respectively (Curtis and Clark, 1950). Therefore, we can say that it is the only monovalent cation which is essential for diverse plant functions and metabolic activities.

2.3 Medicinal plants

Vegetable drugs have been known all over the world for centuries, some at least for 30 centuries (Dastur, 1962). A large number of these are in common use even today. Since disease, decay and death have always afflicted life. The study of disease and its treatment must also have been contemporaneous with the dawn of human intellect. The wild plants provide most of our indigenous medicines and, therefore, deserve more attention. The nutrient status of the soil in which these plants are grown also carries considerable importance. The information about the effect of mineral nutrient on the growth, yield and alkaloid content of medicinal plants is meagre. However, in the following review of literature, some light has been thrown on mineral requirements and alkaloid contents of Datura which is an important medicinal plant.

Sciuchetti (1964) observed the effect of various concentrations (12, 48 and 192 mcg) and frequency of application of gibberellic acid (GA) on growth and metabolic processes of Datura stramonium. The optimum increase in height was recorded with the maximum (192 mcg) dosage of GA. Growth was faster in GA treated plants. The total dry weight of plants was significantly affected by dosage and age. He also noted that alkaloid concentration was reduced in the plant organs at the higher dosage level of GA, decrease percent of chlorophyll in the leaves.

James and Sciuchetti (1964) noted the effect of gibberellic acid and B 995 on Datura innoxia. Thirty three day old Datura innoxia seedlings were treated weekly for a period of 4 weeks with 50 mcg of GA, 100 to 1000 ppm, solution of B 995 or combinations of both. The GA treated plants showed an increase in height and dry weight and generally a decrease in alkaloid concentration. Plants treated with the combination of both were closely matching those treated with GA alone while plants treated with B 995 closely resembled control, except for a decrease in alkaloid concentration in roots at the last harvest.

Sciuchetti and Iturrian (1965) studied the effect of dimethylsulfoxide (DMSO) and B 995 on growth and metabolic products of Datura innoxia. Foliage of Datura were sprayed

with 2 per cent solution of DMSO and B 995 or a combination of both. DMSO sprayed plants resembled control in habit. At the final harvest minor increase was recorded in alkaloid concentration. However, the total alkaloid content per plant was similar to the controls. A reduction in the total chlorophyll content of the leafy tops was also noted. On the other hand, significant reduction in height, growth and plant maturity, total alkaloid content and chlorophyll content were found in B 995 treated plants.

Tikhonov (1967) observed the biogenesis of alkaloids of Datura innoxia. He used C^{14} as a precursor of alkaloids of Datura innoxia. It was observed that acetate, acetone and methylamine are used in the biogenesis of tropane alkaloids. Acetone appeared to be the most specific precursor of the alkaloids. Acetate and methylamine engage in biosynthesis in the roots and acetone in leaves of the plant. He also observed that accumulation of hyoscyamine occurs preferentially in roots while scopolamine got stored in the generative organs of the plant.

Edward (1967) in an attempt to review diverse reactions which are involved in the formation of alkaloids, suggested that alkaloids are the products of non-enzymatic reactions.

However, since the majority of alkaloids are optically active this seems to be highly unlikely. It may be that some steps in the formation of an alkaloid occurs without enzyme, but key steps are almost certainly enzyme controlled. He also reported that many of the reactions are hypothetical and work at the enzymatic level is necessary to establish their validity. About 4,000 different alkaloids have been reported so far and new ones are being discovered at a rapid rate. Therefore, he concluded that it would be an impossible task to isolate and to characterise all the enzymes responsible for alkaloid formation.

Engelstad and Terman (1967) observed the role of fertiliser nitrogen in determining crop yield. They noted that the frequency and magnitude of crop response was generally greater with nitrogen than with phosphorus or potassium.

Libizov et al. (1968) studied the raw materials for the production of hyoscyamine and atropine. They noted that the seeds and pericarp of Datura metel contain 0.5 - 0.7% scopolamine.

Shah and Saoji (1968) studied the effect of plant hormones on growth and alkaloid content of Datura metel Linn. Aerial parts were sprayed saparately with 100 ppm aqueous solution of GA, IAA and IPRA (Indol-3-yl propionic acid).

The stem and roots of GA treated plants showed reduction in alkaloid content. All the parts of IPRA group and the roots of plant treated with IAA showed increase in the percentage of alkaloid.

Marijan and Oz̄imic (1968) observed that influence of gibberellic acid, some microelements and tryptophan on the formation of alkaloids in Jimson weed. A field experiment was carried out from seeds pre-soaked for 24 hours in a 0.02% solution of K salt of GA. leaves were picked for analysis 41-45 days after transplanting. They noted that GA alone greatly increased the total alkaloid content. In general, alkaloid was increased by the microelements. Tryptophan also increased the alkaloid when combined micronutrient and GA was used on the same level as with GA alone except in the case of Zn and Cu. It was also noted that Zn alone had no effect. However, combined with GA, it greatly increased the alkaloid content.

Salonen (1968) studied the effect of urea used as nitrogen fertiliser. He used urea (46% N) and calcium nitrate (15.5% N) in 18 field trials throughout finland. The urea was definitely inferior to calcium nitrate; but the difference was reduced with higher rates. Urea competed best with calcium nitrate when applied to leys.

Cossen (1968) observed some aspects of the alkaloids of Datura tatula. It was noted that maximum alkaloid content, particularly that of scopolamine, was dependent on the growth stage of the plant and also on illumination.

Cossen (1969), In another study, reported the influence of light on ontogenetic variation of scopolamine and hyoscyamine content in the leaves of Datura metel. Datura grown in completely artificial conditions was influenced by day length and intensity of light during ontogenesis of the plant. The formation of alkaloids, long days and intense light promotes the accumulation of scopolamine.

Zielinsk and Szepezynska (1969) studied the alkaloid of Datura ceratocoula. The leaves, stem, roots, seeds and pericarp of Datura ceratocoula contain atropine as well as scopolamine. The percentage content of alkaloid in the leaves increased in the early stages of maturation of the seeds.

Nelvin (1969) studied the alkaloids of the genus Datura. Aerial parts of Datura condida yielded, besides other alkaloids, scopolamine and atropine. Leaves of the South American plants, cultivated in Hawaii, contained the same spectrum of alkaloids but different considerably, in some cases, from the typical plants in their total alkaloid content and in the amount of scopolamine present.

Magdalena et al. (1969) worked out the incorporation of $^{14}CO_2$ in the alkaloids of Datura stramonium. The radioactivity of the total alkaloids of Datura stramonium enhanced with increasing exposure to $^{14}CO_2$. The ratio of activity in shoot to that in root, enhanced as the exposure was increased. In the roots, the ratio of activity of hyoscyamine to that of scopolamine was more than in shoots.

Masao et al. (1970) observed the growth and alkaloid production of Datura tissue culture. They obtained callus tissue from the stems and seeds of Datura stramonium. Yeast extract increased alkaloid content and atropine, scopolamine and some other alkaloids were also reported.

Sinha and Verma (1970) studied the influence of gibberellic acid on growth and alkaloidal content of Datura innoxia. Plant were treated with a single or double spray of 100 and 1,000 ppm GA at various stages of growth. The growth, alkaloid percentage and the total alkaloid content of leaves and roots were generally more in the GA treated plants. The double treatment at various stages of growth further enhanced the alkaloid content.

Balbaa et al.(1970) noted the alkaloid content of Datura suaveoleus. The alkaloid content of different organs of the plant before, during and after flowering were studied. The

maximum alkaloid content (2.37%) was found in stem while the lowest (0.318), in mature leaves. Irrigation at 20 days interval recorded the maximum alkaloid content. The alkaloids scopolamine, meteloidine and traces of hyoscyamine were identified. They also described a new method for the isolation of meteloidine alkaloid.

Giuseppe and Vannini (1970) studied the influence of 2,3,5 triiodobenzoic acid (TIBA) on the increase of alkaloid of Datura stramonium. The effect of TIBA in concentrations of 50 and 100 ppm delayed flowering and induced morphological changes mainly in leaves. Plant growth, expressed as fresh and dry weight, decrease both in roots and particularly in aerial parts of the treated plants. They concluded that since alkaloid concentration was increased in the roots and stem and decreased in leaves of treated plants, TIBA seems to interfere with the transport of alkaloids from the roots to the leaves.

Prabhakar et al. (1971) studied the utilisation of several species of wild Datura from various parts of India for commercial production of hyoscine. They investigated the feasibility of utilising Datura species growing as weeds in sub-Himalayan tract of Jammu and Kangra, for the production

of hyoscine. They recommended that the seeds of Datura innoxia as well as leaves of Datura metel may be exploited economically for this purpose.

Mital and Issar (1971) undertook germination studies in Datura innoxia. They established suitable conditions for getting a high percentage of seed germination in Datura innoxia. Seeds placed on moist filter paper, exposed to alternate temperature of 20°C for 16 hrs and 30°C for 8 hrs gave maximum germination (97%) within 3 weeks. Seeds sown in early April or October also gave high (90-91%) germination percentage.

Szepezynska (1971) compared the atropine and scopolamine content in Datura species. After electrophoretic separation of the alkaloids, atropine and scopolamine were determined colorimetrically in three species of Datura at various stages of their vegetative growth.

Singh (1972) noted the effect of butyl alcohol on the morphology of Datura innoxia (Mill). Seeds of Datura innoxia were separately treated with 6% - 9% and 12% aqueous solutions of n-butyl alcohol for 1 hr at room temperature following by washing with water and exposure to 45°C for 30 minutes. In the second set, the above treatment was given in reverse order. Controls were soaked in water for 1 hr at room

temperature. The seeds were then sown in the field. He noted that the plants raised from treated seeds grew more vigrously with larger leaves, flowers and capsules when compared with control. The morphological characteristics of plants raised from seeds treated with 9% n-butyl alcohol followed by exposure to 45°C were comparatively more pronounced and their alkaloid content was higher than in controls.

Pushpa and Nag (1972) studied the effect of phenylalanine and tyrosine on growth and alkaloid production by Datura metel L. grown in tissue culture. The unorganised static tissue cultures of Datura metel showed the presence of atropine, choline and hyoscine besides 5 other unidentified alkaloids. On transfer to singly supplemental phenylalanine and tyrosine media. The tissues showed considerable increase in alkaloid content in both instances only atropine was confirmed, along with 3 other unidentified alkaloids, in tissue grown on amino acid added media.

Gupta and Gibson (1972) in their studied the protein-alkaloid relationship in Datura stramonium. Sterile root cultures were grown for 2 weeks in a nutrient solution containing chloramphenicol, cycloheximide and puromycin. They separated the alkaloids hyoscyamine and scopolamine by TLC, assayed it spectrophotometrically and also estimated the

the total nitrogen content. Of the three inhibitors tested, only puromycin caused an increase in hyoscyamine production and in the incorporation of proline into it. They claimed that this is the first relationship demonstrated between protein synthesis and alkaloid synthesis.

Gupta et al. (1972) studied the neutron activation analysis of Datura stramonium var. tatula. They reported that the seeds have an extraordinarily high potassium content compared to other plant parts and seeds of other plants. They concluded that this may affect the metabolic processes and also in the germination of seeds.

Evans and Lampard (1972) studied the alkaloids of Datura suaveoleus and noted that it shows distinct difference compared with other Datura species. Aerial parts contain, in addition to hyoscine, apohyoscine, norhyoscine, atropine and noratropine. On the other hand, the roots contain hyoscine, meteloidine and atropine.

Czabajski et al. (1973), in their study on cultivation of Datura innoxia reported that yield of scopolamine increases with the application of NPK fertilisers. However, they reported that the fertiliser response was variable in 3 test years.

Gupta et al. (1973) noted the distribution of total alkaloids and their major components in various parts of Datura metel var. fastuosa at different stages of growth. They also noted that there was some direct link between growth stage and its alkaloid content. The maximum alkaloid content in the leaves was found before flowering. At later stages they reported a continuous decrease. They recommended that the best harvest time of leaves was when the plant was 4-5 months old. Precipitation lowered the alkaloid content. Mature leaves of medium size had maximum percentages of hyoscine and hyoscyamine both in leaves and roots which are however, not constant at different stages of growth. Hyoscine was the principal alkaloid upto preflowering stages. Later, the hyoscyamine content increased. They also noted a direct relationship between stage of maturity of the fruit and its alkaloid content. Very young fruits gave maximum percentage of alkaloids.

Nowacki and Waller (1973) observed the alkaloid transport in plants. They also noted the role of alkaloids in plants and specially the translocation of alkaloids of various varieties including Nicotiana tabacum and Datura metel, were fed with species specific and foreign alkaloids. Generally all alkaloids were rapidly translocated within a few hours after administration. The species foreign compounds remained at the site while species specific alkaloids were translocated from old to young leaves.

Khyranin (1973) studied the effect of gibberellin on the process of synthesis of alkaloids and chlorophyll in some drug plants, including Atropa belladonna, Datura stramonium and Datura inermis etc.

De-Miguel and Soriano (1974) studied the breakage of dormancy in Datura ferox seeds as an effect of water absorption. Seeds did not germinate when they were incubated intact and in darkness or on dry storage. If allowed to absorb water vapour for 3-4 weeks at 20°C, they lost dormancy, germinating even when incubated in darkness.

Walleria et al. (1974) undertook the quantitative determination of tropine alkaloids and investigated the scopolamine and hyoscyamine content in Datura innoxia. They noted the ratio of scopolamine and hyoscyamine content of fresh plant juice of individual organs during four different growth periods. The most favourable season for harvest of the crude drug was indicated with respect to scopolamine production. The maximum alkaloid content was in leaves during flowering and in fruits at ripening.

Czabajski (1974) studied the NPK requirements of Datura innoxia and noted that nitrogen gave maximum response when it was supplied with phosphorus and potassium, NPK fertilization and liming of the soil did not change the

scopolamine and atropine content; but the highest yields of the compounds were obtained when full fertiliser and lime were applied. He also noted that the plant's requirements for nitrogen and phosphorus were considerable.

Leokadia et al. (1975) noted the composition of oil from the seeds and total alkaloid content in the leaves of Datura stramonium, 3 plots were organised, (i) without mineral fertilisation (control), (ii) NPK dose (60 kg N/ha, 48 kg P_2O_5/ha and 72 kg K_2O/ha) and (iii) double of the above NPK dose. They reported little influence of mineral fertilisation on the yield and chemical composition of Datura oil and the total alkaloid content in leaves, but yield of the crop, interms of seeds and leaves, was significantly increased by applying NPK fertiliser.

Sinha and Verma (1975) studied the influence of indol-3 acetic acid (IAA) on growth and alkaloid content of Datura innoxia. Twenty groups of Datura innoxia were given a single or double treatment of 100 and 1,000 ppm of IAA respectively at different stages of growth. Growth, as well as alkaloid content, was generally better in treated plants than in the controls. Comparatively, double treatment proved more effective.

Nowacki et al. (1975) noted the effect of availability of nitrogen on alkaloid synthesis in solanaceous plants. The effect of nitrogen availability on alkaloid synthesis in 3 species (Nicotiana rustica, Datura stramonium and Solanum tuberosum) was investigated with increased N fertilisation. The N content in the dry matter increased while the carbohydrate content decreased. The Nicotiana and Datura alkaloid increased while the content of the Solanum glycoalkaloid in the dry matter decreased.

Gabr et al. (1975) noted the change in the absolute amount of alkaloid in Datura metel treated with certain growth regulators. Seedlings were sprayed, or the seeds were soaked before sowing, with solution of 2-chloroethyl trimethyl ammonium chloride. GA_3 or B-9 (n-dimethyl amino succinamic acid). The treatments caused a decreased or increased in alkaloid content (measured as hyoscine and hyoscyamine) depending on the growth substances used its concentration, method of it application, the organ and finally the age of the plant.

Gabr et al. (1975) noted the effect of 2-chloroethyl trimethyl ammonium chloride upon growth of Datura metel L. It was applied by spraying seedlings or by soaking the seeds before sowing. Both spray as well as seed soaking caused an increase in plant height, number of branches/plant, number of leaves/plant and dry weight/plant.

Lewis (1975) used ^{15}N and ^{14}C to study the role of the leaf in nitrogen nutrition of seeds of Datura stramonium. $^{15}N - NO_3$ feeding via transpiration stream and simultaneous feeding of ^{14}C via photosynthesis to a leaf fruit system indicated that glutamine was the prime recipient of photosynthetically reduced nitrogen in leaf. Analysis of petiole and seeds showed that glutamine supplied the seeds with most of the reduced nitrogen required for amino acid synthesis. Carbon and nitrogen estimation in leaf did not appear to be directly related with serine and asparatate and not glutamine receive the heaviest initial ^{14}C label.

Gwiazdzinska and Zakrzewski (1975) studied the correlations between total alkaloid content in leaves and roots of nightshade. Several dried extracts from Datura leaves and roots were obtained by them. An appropriate selection of leaves and roots was performed to select crude drugs which have different total alkaloid content. They reported that an application of ethanol (70%) gave a preparation with higher total alkaloid content. They also recommended that the requirements for the extraction of alkaloids should be changed if ethanol 40% is to be used. They were of the view that the method of the Polish pharmacopeia IV for alkaloid determination in Datura should be either replaced or modified if ethanol (70%) is to be used for the extraction procedure.

Padula et al. (1976) determined total alkaloids and scopolamine quantitatively in Datura ferox. The occurance and amount of total alkaloids and scopolamine in different part of the plant were investigated. They reported that alkaloid content ranged from 0.02 - 0.52 g of total alkaloid and 0.0029 - 0.32 g of scopolamine/100 g of dried material.

Bevezegovskaya et al. (1976) noted that biological rhythms in tissue culture. Datura innoxia and Scopolia stramonifolia tissue in static and suspension cultures showed similar annual rhythm with irregular growth and alkaloid production depending on the season. They noted that spring was the most productive season in alkaloid biosynthesis. They obtained a negative correlation between increase in biomass and alkaloid synthesis. Seasonal changes were also observed for several indices of nitrogen metabolism such as level of total nitrogen and protein based on their mitotic activity. They also indicated the practical significance of the work for the pharmaceutical industry, claiming tissue culture as a source of a new type of drug.

Afridi et al. (1977), while working on the effect of different levels of nitrogen on the growth and alkaloid content of Datura innoxia, noted that plant length, number of branches, fresh and dry weight and alkaloid content of leaves were signi-

ficantly higher in the treated plants than in the control. They reported that 60 kg nitrogen/ha, supplied as urea, proved optimum under local conditions.

Bhattacharya et al. (1977). Studied the triterpenoids from Datura metel fruits. Daturaolone and daturadial, the 2 closely related triterpenoids belonging to the oleane series, were reported from the fruits of Datura metel.

Grewal et. al.(1977) studied the effect of various hormones on growth, differentiation and alkaloid formation in Datura innoxia apical meristum culture in vitro. Static cultures established from the apical tip meristum grown on media containing different hormones were tested for alkaloid formation, BA (benzyladenine) and NAA, each at concentrations higher than 10^{-6}M and BA at $3x10^{-7}$M when combined with 10^{-6}M NAA and 2,4D 10^{-7}M inhibited hyoscyamine formation. BA at the same concentration when combined with 10^{-7}M IAA and 10^{-6}M IBA (indole butyric acid) favoured hyoscyamine formation.

Khaleque et al. (1977) reported the isolation of atropine and three other new alkaloids from Datura fastuosa seeds namely fasturine ($C_{17}H_{23}O_3N$), fastudine ($C_{27}H_{20}O_3N_2$), and fastusidine ($C_{16}H_{21}O_3N$).

Pandita et al. (1977) reported that Datura innoxia also behaved as a perennial herb. They observed that the perennial plants in no way differed from the annual plants. They noted that the percentage of alkaloids in seeds was 0.29 and 0.23 in 1972 and 1973 respectively.

Schell et al. (1977) studied the influence of mineral nutrition on the amount of tropane alkaloids in different organs of the plant. The data suggested a function of mineral nutrition and the time of plant harvesting. Highest amount of these alkaloids was noted in roots, leaves and flowers harvested with young fruits grown with nitrogen. They also reported a fourth unindentified alkaloid in the flowers harvested with young fruits.

Singh and Kaul (1977) studied the effect of temperature on seed germination in Datura species. Data on germination of Datura innoxia, Datura metel and Datura stramonium was given for temperature ranging between 20 and 35°C. They recommended September/October as the suitable time from direct seeding of Datura innoxia and Datura stramonium. On the other hand, March/April was the best seeding time for Datura metel.

Abou-Zied et al. (1978) reported the effect of B-9 (succinic acid 2, 2 dimethyl hydrazide) applied by spraying

or soaking the seed, for 12 hrs. They noted that plant height and branch number increased with 250 ppm spray application and decreased with 4,000 ppm spray application. Soaking increased these parameters at all concentrations with the greatest stimulation at 1,000 ppm, B-9 (either when used for soaking or sprayed) generally lowered the dry weight of leaves, stem and roots.

Afaq et al.(1978) studied the effect of different levels of phosphorus on growth and alkaloid content of Datura innoxia. Application of 0,30, 60 and 90 kg P_2O_5 per hectare significantly increased the number of branches, fresh and dry weight of shoot and alkaloid content of leaves in comparison with the control. The application of 90 kg P_2O_5 per hectare proved optimum for all the characteristics.

Afridi et al. (1978) studied the effect of three levels of nitrogen and phosphorus (each @ 0,30,60 kg/ha) individually and in combination on total yield of dill (Anethum sowa L.) fruits, percentage of oil and carvone content, these were 62.4, 98.9 and 3.7% higher respectively, in the plants receiving 30 kg than in those receiving 60 kg N/ha. A dose of 30 kg P_2O_5 /ha proved optimum for total fruit yield among phosphorus levels which was 8.5% higher than in control (P,0). Regarding the interaction of nitrogen

and phosphorus, the combination $N_{90}P_0$ proved best for both total yield and percentage of oil.

Gabr et al.(1978) treated the seeds or seedlings with gibberellic acid (0, 25, 100 or 400 ppm) and noted the effect on the growth of Datura metel. Gibberellic acid was applied by soaking the seeds before sowing or as a foliar spray. They observed that highest leaf dry weight at the fruit ripening stage was given by 400 ppm spray of GA_3 while soaking the seeds with the same concentration of GA_3 gave the maximum stem and root dry weight and increased branching. They were of the view that an increase in leaf dry weight might be due to an increase in total leaf area per plant. Seed soaking appeared to stimulate root growth more than did foliar spray while foliar spray seemed to promote leaf growth.

Lewis and Probyn (1978) studied the effect of nitrate feeding levels on the pathway of nitrogen incorporation into photosynthesizing leaf metabolism of Datura stramonium, leaves fed $K^{15}NO_3$ at 25 µg N ml-1 and 200 µg N ml-1 show that main route of newly reduced nitrogen is to glutamated at the low N feeding level. At the high N feeding level the amino nitrogen of glutamine showed higher ^{15}N enrichment than the amino nitrogen. At the low N-feeding level, the opposite was true. Feeding of the glutamine at the 25 and

200 ug N ml-1 level produced ^{15}N enrichment of the leaf amino acids . The prime routing being to glutamate synthase at both feeding levels. It is possible that Datura stramonium leaves both the glutamate dehydrogenase and glutamine synthetase/GOGAT pathway were simultaneously working, the former route being affected at low nitrogen feeding levels while the latter, at high N-feeding level.

Ruminska and Gamal (1978) reported the effect of nitrogen on growth, yield and alkaloid content of Datura innoxia. Plants grown in pots with various levels and forms of nitrogen fertilisers showed that as the nitrogen rose from 150 to 450 mg per pot, growth was enhanced proportionally. However, it decreased at higher rates. Growth was greater when plants received ammonium sulphate or urea than when calcium nitrate was given. The total alkaloid content was unaffected by the percentage of total nitrogen in the plants.

Ruminska et al. (1978) noted that pre-soaking seed treatment in solution of GA (500, 1,000, 1,500 or 2,000 ppm) improved the germination of 7 species. They reported that Datura innoxia, Atropa belladona, Digitalis lanata and lavender responded particularly well, germination being accelarated as well as increased. Optimum GA_3 concentrations

were 1,000 and 1,500 ppm respectively, for the two crops. The maximum response in lavender to treatment was observed after 21 days from soaking.

Mechler and Kohlenbach (1978) reported that the alkaloid content of the leaves of haploid Datura plants was less than in diploid Datura plants in the vegetative state, but the alkaloid pattern was same. Diploids had as their main alkaloid scopolamine together with hyoscyamine and meteloidine. The alkaloid content of haploids and diploids depended on the development of the plants. Diploids had the highest level in the vegetative state, rapidly, decreasing when the plants flowered and on the development of fruits whereas, haploids showed smaller decrease.

Yadrov et al. (1978) studied the effect of copper, manganese and cobalt on the productivity of isolated tissue culture of Datura innoxia. They reported that nutrient medium containing 0.1 mg/l Cu, 89.2 mg/l Mn, and 0.1 mg/l Co was optimal for the biosynthesis of tropane alkaloids. Tissue culture in darkness accumulated all the major and minor elements in the nutrient medium, except Zn.

Mubarak and Hussain (1979), in their study on biochemical inhibition exhibited by Datura innoxia seeds,

noted that the possible factor responsible for dormancy may be the hard test and biochemical inhibitors in the seeds. The artificial rain-drip, water extract from whole seed, seed coat and cotyledon proved to be inhibitory for germination and early growth of its own seeds as well as of other seeds tested. According to them, the whole seed and the seed coat extract were more toxic than the cotyledon extract. They were of the opinion that the presence of inhibitors may be one of the possible causes of delayed, irregular and reduced seed germination.

Azimuddin et al. (1979) noted the effect of gibberellic acid on growth and development of Datura metel. They considered four concentrations of GA (5, 10, 50, and 100 ppm) and applied as foliar spray. They noted that plant height, internodal length and number of leaves increased significantly by the application of GA. However, base diameter of the plants and breadth of the leaves decreased slightly. It was also noted that GA treatment brought about early flowering and increased the number of flower buds.

Wasiuddin et al. (1979) studied the effect of five levels of basal nitrogen (0, 30, 60, 90 and 120 kg N/ha) on growth of Cichorium intybus Linn. at two stages and on

vegetative yield at harvest. They reported that most of the growth and yield characteristics were affected significantly by nitrogen treatments of which 90 kg N/ha proved optimum. From the point of view of drug productivity they were of the view that the only two important characteristics were (i) fresh weight of shoot and (ii) dry weight of root. For both of these, 60 kg N/ha gave equally good results. Hence, for ensuring economic yields of the drug, this dose may be safely recommended.

Amalraj (1981) reported that Datura a common medicinal weed yielding scopolamine, had 10 species prevalent in India. Three of these, Datura stramonium, Datura innoxia and Datura metel, were more important as drug plants. Scopolamine appeared to be the major alkaloid present in all parts of the plant though 10%, 20% of Hyoscymine was also present.

Janine et al. (1981) noted the influence of sodium chloride on tropane alkaloid content of Datura innoxia grown under controlled conditions. They analysed root, stem and leaf for K^+, Na^+, Ca^{2+} and Cl^- content and measured Hyoscyamine and scopolamine levels and reported a moderate increase in the response to salt stress.

Gerogi and Yankulov (1981) reported the changes in herb yield and alkaloid content of diploid and tetraploid

forms of Datura innoxia. The study was conducted during 1974-75 on three seeding dates (April, May and June). Highest herb and alkaloid yield was produced under conditions existing in the Kazauluk (Bulgaria) plain following seeding in mid-April (11-15). Later seeding preserved high alkaloid content but the herb yield was lower. They also noted that tetraploid forms were superior to the diploids in atropine content.

Yakulov and Gerogi (1982) reported the morphogenetic variability of the concentration of scopolamine and atropine in tetraploid forms of Datura innoxia. It was noted that tetraploid level of Datura innoxia contributed to an increase in the root system by 123.6% and in the stem by 147.5%, while decreasing the fruit yield by 33.6%. The tissue of the tetraploid were poorer in dry matter only 1 kg dry herb being obtained from 64 kg fresh mass; but the quantity obtained in diploids was 5.1 kg. The scopolamine synthesis was retained but in tetraploid synthesis was notably more intensive. Atropine synthesis was also more intensive. The data for synthesis and localization of alkaloids in tetraploid forms elucidate their advantage in cultivation of Indian stramonium for scopolamine.

Wasiuddin et al.(1982) studied the growth and yield of Kasondi (Cassia occidentalis) at Aligarh (Uttar Pradesh) in relation to the effect of nitrogen and phosphorus applied separately in two field trials. In the first experiment, four levels of nitrogen (0, 10, 20 and 30 kg N/ha) were applied to the soil to which 40 kg P_2O_5/ha and 30 kg K_2O/ha were added uniformly. In another trial on phosphorus, four levels (0, 20, 30 and 40 kg P_2O_5/ha) were applied as super phosphate. Simultaneously, nitrogen as urea and potassium as muriate of potash were applied uniformly in all the plots at the rate of 20 kg N/ha and 30 kg K_2O/ha respectively. They noted that 30 kg each of N and P_2O_5 were optimum for growth and yield of C. occidentalis.

Samiullah et al. (1982) conducted a field trial to study the effect of varying levels of phosphorus (0, 50, 100, 150 and 200 kg P_2O_5/ha) on the yield of linseed (Linum usitatissimum L.) at Aligarh. Nitrogen and potassium, at the rate of 100 kg N/ha and 25 kg K_2O/ha, respectively, were applied uniformly in all the plots. They reported that basal phosphorus increased all yield characteristics significantly. However, the number and weight of seeds per capsule were maximum in P_{200}. It was, however, concluded that P_{150} was the economical dose for linseed cultivation under local conditions.

Eva et al. (1983) noted the changes in total alkaloid content in tissue culture of Datura innoxia in various cultural situations. During the growing period, the total alkaloid content of callus tissue of different origin (root, leaf, corolla, gynoecium) was examined simultaneously with biomass production. The alkaloid content was high in the first 2 weeks of growth and then it slowly decreased when the growth of the tissue became intensive. At the end of the growing period, when stabli-zation of the tissue set in, it rose again. Kinetin inhibited the alkaloid production. Under the influence of 2, 4-D, however, alkaloid content suddenly rose. Light increased the alkaloid content of the callus tissue obtained from roots and leaf 2-fold compared with the cultures grown in the dark.

Megaw and Woolley (1983) studied the role of hygroline in tropane alkaloid biosynthesis. Datura innoxia Mill. plants were fed via the roots with (2 - ^{3}H; 2 - ^{14}C) hydroline. After 6 days the plants were harvested and the root alkaloid isolated. It was reported that hygroline was not an immediate precursor of tropane moiety.

Lindsey and Yeoman (1983) reported the relationship between growth rate differentiation and alkaloid accumulation

in cell culture of 7 solanaceous species (Datura innoxia, Datura stramonium, D. clorantha, Hyoscyamus niger, Atropa belladonna , Solanum dulcamara, Solanum nigrum). Evidence was obtained for an inverse correlation between the growth phase on one hand and alkaloid content and organisation on the other. The alkaloid content dropped concomitant with a decrease in growth rate.

Afridi et al. (1983) studied the effect of nitrogen, phosphorus, and potassium on the growth and yield of fennel. The effect of three levels of N, P and K (0, 60 and 90 kg/ha each of N, P_2O_5 and K_2O), individually and in all possible combinations, on growth characteristics at two stages (110 and 130 days after sowing) and on yield of fennel at harvest was investigated at Aligarh during the winter of 1977 - 78 according to a partially confounded design.

Considering main effects, 90 kg N, 60 kg P_2O_5 and 90 kg K_2O/ha gave the best growth at both stages. Among first and second order interactions, 90 kg N with 90 kg K_2O/ha and 90 kg N with 60 kg P_2O_5 and 90 kg K_2O/ha respectively proved best. Fruit yield was highest in plants receiving 90 kg N or 90 kg P_2O_5 whereas, 60 kg as well as

90 kg K_2O/ha proved equally good. Among first order interaction, 90 kg each of N, P_2O_5 and K_2O/ha gave highest fruit yield.

Afaq et al. (1984) conducted a field experiment at Aligarh on Anethum sowa L. to study the effect of four levels each of nitrogen (0, 40, 80, 120 kg N/ha) and phosphorus (0, 15, 30, 45 kg P/ha) as urea and superphosphate, respectively, in all possible combinations according to factorial randomised block design. Six parameters, namely fresh weight of shoot/plant, dry weight of shoot/plant, number of branches/plant, number of umbels/plant and number of umbellets/umbel were noted at vegetative and reproductive stages. Total weight and yield of fruit were noted at harvest. Various treatments had a significant effect on all the characters studied. Individually 80 kg N and 30 kg P/ha proved optimum for most of the parameters studied at both stages of growth and the combined application of these same doses of N and P gave much more pronounced results.

Baytop and Nushet (1984) reported different percentages of alkaloid in species of Datura, Hyoscyamus and other medicinal plants. Datura metel was the species richest in scopolamine (0.445% in seeds).

Afaq et al. (1985) worked out the leaf nitrogen content of Mako (Solanum nigrum L.) fruits grown in pot culture for prediction of solasodine content. They applied nitrogen at the rate of 0, 0.45, 0.90, 1.35, 1.80 or 2.25 g N/pot at sowing. Leaf N content was estimated at 40 days and solasodine content in mature fruit at 85, 130 and 175 days after sowing. They reported that leaf N and fruit solasodine were both significantly affected, 1.8g N/pot proving optimum. It gave 0.15% more leaf N at 40 days and 0.02, 0.11 and 0.14% more solasodine content than the control at the 3 samplings respectively. Solasodine was significantly correlated with leaf N at $P < 0.05$ ($r = 0.7058, 0.6224, 0.6699$). As low leaf N values indicate low fruit solasodine content, early corrective measures like top-dressing with, or foliar application of , urea, may be undertaken to optimise solasodine production.

Afridi and Khan (1985) studied the growth and productivity of Isubgol (Plantago ovata Forsk) in Western Uttar Pradesh as affected by sowing date. For this purpose, a simple randomised field experiment was conducted on Isubgol. Eight physiomorphological parameters (length, culm number and leaf number/plant, leaf area, fresh and dry weight/plant,

N.A.R and R.G.R.) that contributed to final yield were studied at three stages of vegetative growth. Seven dates at 10 days intervals from 15 Oct. to 14 Dec were considered. A uniform basal dose (70 kg N as urea and 10 kg P as single superphosphate/ha) was added at each sowing. They reported that most of the characteristics were significantly affected by sowing date. They opined that delay in sowing had adverse effect upon the growth pattern of the crop as revealed by almost all parameters studied. Sowing on 25 Oct, promoted best vegetative growth, leading to highest seed yield.

Khan and Afridi (1985) studied the effect of sowing date on yield performance of Plantago ovata Forsk. Seven sowing dates, at 10 days intervals from 15 October to 14 December were selected. Fertilizer was applied at the rate of 40 kg N and 10 kg P/ha uniformly at each sowing. Spikes/plant, length and flower/spike and yield, hectrolitre weight, moisture and ash content of seed was studied at harvest. Sowing date proved crucial for all attributes, including seed yield, the latter being 15 Oct: 985 kg, 25 Oct : 1, 226 kg (optimum) 4 Nov : 1, 067 kg, 14 Nov : 947 kg, 24 Nov : 650 kg, 4 Dec: 305 kg and 14 Dec : 257 kg/ha. Compared with increased to about 10 q/ha (79%) at the last sowing (14 Dec.) due to adverse effect on most of the yield attribute. They concluded

that isubgol could be grown profitably in Western Uttar Pradesh if sown on 25 Oct (or latest by 4 Nov).

Khan et al. (1985) studied the yield and quality of fennel (Foeniculum vulgare Mill) in relation to basal and foliar application of nitrogen and phosphorus. A split-plot yield trial was conducted to study the effect of foliar application of 0 (control), 20 kg N/ha 2 kg P/ha or 20 kg/N+ 2 kg P/ha at low basal level, Viz, 90 kg N + 40 kg P/ha (optimal dose) and 60 kg N + 27 kg P/ha (sub-optimal dose) each with 50 kg K/ha applied uniformly to the field. Spray was applied at flowering stage, 120 days after sowing. Of the two main plot treatments, the optimal basal dose gave better results than the sub-optimal basal dose. Among spray treatments 20 kg N + 2 kg P/ha proved optimum. The combined spray of nitrogen and phosphorus applied to plant grown with sub-optimal basal dose proved better for most of the yield and quality parameters than when applied to plants grown with the optimal basal dose. The percentage of anethole (but not of fenchone) was significantly higher in plants grown with the sub-optimal basal dose. Foliar application of N,P and N + P, on the other hand, decrease the anethole content of oil and increased that of fenchone significantly.

Pazir et al.(1985) reported that oil extracted from the seeds of Datura stramonium , with the help of soxhlet apparatus using petroleum ether (40 - 60°C) as solvent, was purified by treating it with activated charcoal, yield, physicochemical characteristics and chemical composition of the oil were studied and compared with those of other Datura species. This oil was similar to those of the other species and could be used as medicine.

From the foregoing survey of literature, it is clear that although some work has been conducted on the effect of nutrients and other chemicals, but it is confined mostly to the alkaloid content of various species of Datura. However, no in-depth study has been undertaken on the effect of hormones, vitamins and chemicals on growth and yield parameters or enzyme activity. Hence, it is proposed to undertake such a study selecting only one species of Datura, namely Datura metel, on the lines given in chapter III.

C H A P T E R - 3

PROPOSED STUDY

C O N T E N T S

CHAPTER - 3

PROPOSED STUDY

3.1 Introduction

Four pot experiments on Datura metel L. are proposed to be undertaken to determine the effect of various doses and sources of nitrogen on the growth, yield, NPK contents, NRA, chlorophyll and alkaloid content of the plant. The response to supplemental top-dressing and foliar nutrition will also be studied. The effect of seed soaking in various soaking agents, like gibberellic acid, potassium iodide and pyridoxine will also be investigated.

The experiments will be conducted at the Botany Department, Aligarh Muslim University, Aligarh. The results will be analysed statistically according to the design of each experiment. The physico-chemical characteristics of the pot soil will be determined before the start of each experiment.

3.2 Preparation of the pots

Earthen clay pots of size 25 cm (diam.), having about 4 kg of soil mixed with manure will be sterilised by autoclaving. Pots will be arranged according to the design of each experiment.

3.3 Soil characteristics

Before sowing, a small quantity of soil from each pot will be collected and mixed to form a composite soil sample.

Texture, pH, conductivity, available nitrogen, phosphorus and potassium of the soil samples will be determined before the application of chemical fertilizers.

3.4 Seeds

Healthy seeds of more or less uniform size will be selected and surface sterilised with 95 per cent ethyl alcohol and washed thoroughly with de-ionised water before sowing.

3.5 Experiment 1

A pot experiment will be conducted according to a factorial randomised block design on Datura metel during the year 1985-86 (winter). Three sources of nitrogenous fertilizers, namely urea, diammonium phosphate and ammonium nitrate will be applied to the soil to study the growth, yield, NPK, NRA, chlorophyll and alkaloid percentage of the plant. Three doses of urea, diammonium phosphate and ammonium nitrate (50, 100, 200 mg N/pot), will be tested to select the optimum fertilizer requirement and nitrogen source for this plant on the basis of criteria given above (Table 1). In the end, correlations between the nitrogen dose and various parameters will be worked out so as to establish the degree of participation of nitrogen in the manifestation of these characters.

Sixteen replicates of each treatment will be taken. A uniform dose of phosphorus (100 mg P_2O_5/pot) as superphosphate and of potassium (50 mg K_2O/pot) as muriate of potash will be applied before sowing. The scheme of the treatments is given in Table 1.

Table 1: Scheme of the treatments

(Experiments 1 and 2)

S.No.	Source	Nitrogen		
		per hectare (Kg N)	per pot (mg N)	quantity of fertilizer used per pot (mg)
1.	Control	Nil	Nil	Nil
2.	Urea	25	50	107.50
3.	"	50	100	215.00
4.	"	100	200	430.00
5.	Diammonium phosphate	25	50	236.00
6.	"	50	100	472.00
7.	"	100	200	944.00
8.	Ammonium nitrate	25	50	143.00
9.	"	50	100	286.00
10.	"	100	200	572.00

N.B. A uniform basal dose of 50 kg P_2O_5/ha (100 mg P_2O_5/Pot or 625 mg superphosphate/pot) and 25 kg K_2O/ha (50 mg K_2O/pot or 80 mg muriate of potash/pot) will be applied.

3.6 Experiment 2

This pot trial will be started and will be conducted during the summer in the month of April, since Datura metel is biennéal in nature and could be sown in March - April also. The same doses and sources will be tested to note the differences, if any , in growth, yield, NRA, NPK, chlorophyll and alkaloid percentage due to difference in sowing date. The scheme of the treatments will be the same as given in Table 1.

3.7 Experiment 3

This experiment will be conducted during the year 1986-87 according to simple randomised block design. The optimum dose and suitable source of nitrogen obtained from Experiments 1 and 2 will be applied to Datura metel partly through basal application and partly by top-dressing to study the response to the applied. The seeds will be soaked in GA_3 (0.1%), K I (1.0%) and pyridoxine (0.02%) to study the effect of these chemicals. The aim of this experiment will be to find out wheather top-dressing of nitrogen is advantageous over its application at sowing since the crop is of long duration and nitrogenous fertilizers are leached down if they are applied at the time of sowing. In this trial, nutrient content, yield parameters, and alkaloid percentage will be considered.

Table 2. Scheme of the treatments.

(Experiment 3)

S.No.	Soaking agents	Mode of nitrogen application	
		Basal (N)	Top(N)
1.	Unsoaked	Full	nil
2.	"	3/4	1/4
3.	"	1/2	1/2
4.	Gibberellic acid	Full	nil
5.	"	3/4	1/4
6.	"	1/2	1/2
7.	Pyridoxine	Full	nil
8.	"	3/4	1/4
9.	"	1/2	1/2
10.	Potassium iodide	Full	nil
11.	"	3/4	1/4
12.	"	1/2	1/2

N.B. A uniform basal dose of 50 kg P_2O_5/ha (100 mg P_2O_5/pot or 625 mg super phosphate/pot) and 25 kg K_2O_5/ha (50 mg K_2O/pot or 80 mg muriate of potash/pot) will be applied.

3.8 Experiment 4

During the year 1986-87 another study will also be conducted to study the efficacy of foliar application of nitrogen. This experiment will also be based on simple randomised block design. The seeds will be soaked in GA_3, pyridoxine and potassium iodide. The aim of the experiment will be to study the feasibility of foliar nutrition and fertilizer economy. Only N content, yield characteristics and alkaloid percentage will be considered in this trial. For this purpose, the schedule of fertilizer application is mentioned in Table 3.

Table 3 : Scheme of the treatments.

(Experiment 4)

S.No.	Soaking agents	Basal (N)	Spray treatments per hectare (N kg)	Spray treatments per pot (N mg)	Spray treatments fertilizer used per pot (Urea mg)
1.	Unsoaked	Full	Nil	Nil	Nil
2.	"	3/4	10	20	43
3.	"	1/2	"	"	"
4.	Gibberellic acid	Full	Nil	Nil	Nil
5.	"	3/4	10	20	43
6.	"	1/2	"	"	"
7.	Pyridoxine	Full	Nil	Nil	Nil
8.	"	3/4	10	20	43
9.	"	1/2	"	"	"
10.	Potassium iodide	Full	Nil	Nil	Nil
11.	"	3/4	10	20	43
12.	"	1/2	"	"	"

N.B. A uniform basal dose of 50 kg P_2O_5/ha (100 mg P_2O_5/pot or 625 mg super phosphate/pot) and 25 kg K_2O/ha (50 mg K_2O/pot or 80 mg muriate of potash/pot) will be applied.

3.9 Sampling technique

One plant from each replicate will be taken randomly at the time of sampling which will be done at various growth stages.

3.9.1 Vegetative characteristics

The following vegetative characters will be studied:

1) Shoot length/plant.
2) Root length/plant.
3) Leaf number/plant.
4) Leaf area/plant.
5) Branch number/plant.
6) Fresh wt/plant
7) Dry wt/plant

3.9.2 Reproductive characteristics

The following characteristics will be studied at flowering, fruiting and harvest:

1) Bud number/plant
2) Flower number/plant
3) Fruit number/plant
4) Seed number/fruit
5) Yield/plant

3.9.3 Nutrient content

The following data will be obtained by leaf analysis:

1) Percentage of nitrogen in leaves
2) Percentage of phosphorus in leaves
3) Percentage of potassium in leaves

3.10 Leaf analysis

Three plants from each treatment will be wiped free of any adhering dust. Root will be severed away and the samples will be dried for 24 hrs. in an oven at 80°C. Fully mature and expanded leaf blades will be detached from the shoots, powdered and passed through a 72 mesh screen. The powder will be stored in polythene bags after properly labelling.

The leaf powder will be kept at 70°C overnight before being digested and analysed for its nitrogen, phosphorus and potassium content, according to the method of Lindner (1944).

100 mg of dried leaf powder of each sample will be carefully weighed and transferred to a 50 ml Kjeldahl flask. It will be wet ashed in 2 ml of chemically pure sulphuric acid. To allow for complete reduction of nitrates present in the plant material by the organic matter itself, digestion will be continued for about 2 hours. Dense fumes will be given off at

this stage and the contents will turn black. The flask will be cooled for about 15 minutes. After cooling, 0.5 ml of chemically pure 30 per cent hydrogen peroxide will be added dropwise and the solution will be heated again till the colour of the solution changes from black to light yellow. After heating for about 30 minutes, the flask will be kept for cooling for about 10 minutes to get the clear and colourless extract. At this stage, 3 or 4 additional drops of 30 per cent hydrogen peroxide will be added in like manner, followed by gentle heating for about 15 minutes. Care will be taken at the time of adding hydrogen peroxide because its excess might oxidise ammonia in the absence of organic matter. The digested peroxide material will be diluted with double distilled water and transferred with three or four washings to a 100 ml volumetric flask and the volume will be made up to the mark with double distilled water. Suitable aliquots will be taken from these sulphuric acid-peroxide digested samples for determining nitrogen, phosphorus and potassium. The methods employed for the estimation of these elements are briefly discussed below.

2.10.1 Nitrogen

The nitrogen content of the samples will be estimated according to Lindner (1944). A 10 ml aliquot of the digested material will be taken in a 50 ml volumetric flask and the

excess of acid will be partially neutralised with 2 ml of 2.5 N sodium hydroxide. To this, 1 ml of 10% sodium silicate will be added to prevent any turbidity. After making up the volume, 5 ml aliquot of this solution will be taken in 10 ml of graduated test tube and 0.5 ml of Nessler's reagent will be added drop by drop. Double distilled water will be added to make the volume upto 10 ml and the contents will be allowed to stand for about 5 minutes for maximum colour development. The solution will then be transferred to a colorimetric tube and the optical density will be measured at 525 nm on a "Spectronic 20" colorimeter. A blank will be run with each set of determinations and the amount of nitrogen in the aliquot will be read from a calibration curve obtained using known dilutions of a standard ammonium sulphate solution.

2.10.2 Phosphorus

Total phosphorus in the sulphuric acid-peroxide digest will be estimated by the method of Fiske and Subba Row (1925).

A 5 ml aliquot will be taken in a 10 ml graduated test tube and 1 ml molybdic acid (2.5 per cent ammonium molybdate in 10 N H_2SO_4) will be added with care, followed by 0.4 ml of 1,2,4- amino nephthol sulphonic acid. The colour will change to blue. Double distilled water will be added to the blue

solution to make the volume upto 10 ml. The thoroughly stirred solution will be kept to stand for about 5 minutes and then transferred to a colorimetric tube and the optical density will be read at 620 nm. The standard curve will be prepared by using known dilutions of a standard monobasic potassium phosphate solution.

2.10.3 Potassium

Potassium will be estimated flame photometrically. A 1 ml aliquot will be taken and after proper dilution it will be read at 768 nm, using potassium filter. A blank will be run side by side. The reading will be compared with the calibration curve plotted for different dilutions of a standard potassium sulphate solution.

3.11 Estimation of nitrate reductase activity (N R A)

Nitrate reductase activity (NRA) in leaves will be estimated at various growth stages viz. 25, 35, 45 and 55 days after sowing. Random leaf samples from each pot will be taken. These leaves will be cut into small pieces and NRA will be determined according to Jaworski's method (1971).

500 mg leaf pieces will be weighed and placed in polythene vials. 2.5 ml phosphate buffer and 0.5 ml of 0.2M

potassium nitrate solution will be added followed by addition of 2.5 ml of 5 per cent isopropanol alcohol. At least 2 drops of chloramphenicol will also be poured to avoid bacterial growth in the test medium. These vials will be incubated for 2 hrs in dark at 30°C.

Colour development:

0.4 ml of test extract will be taken in a test tube in which 0.3 ml of sulphanilamide and NED Hcl will be added. The set will be left for 20 minutes for maximum colour development. A pink colour will be developed which will be diluted upto 5 ml with double distilled water and read at 540 nm using Spectronic 20" colorimeter.

A blank consisting of 4.4 ml of double distilled water plus 0.3 ml each of sulphanilamide and NED Hcl will run simultaneously.

A standard curve will be plotted by taking various dilutions of a standard potassium nitrite solution. The readings of test extracts will be compared with this calibrated curve and NRA will be calculated in μ mol No_2^- produced per hour per gram of fresh leaf tissue.

3.12 Estimation of tropane alkaloids:

Alkaloid will be estimated by the method of Colby and Beal (1952).

To 1 g powdered material, 1 ml of 95% ethanol and 0.1 ml of 10% ammonium hydroxide will be added for moistering. Then, 5 ml of chloroform will be added and the mixture heated to boiling for 3 minutes. The material will be transferred to a miniature percolator previously plugged with small amount of cotton wool soaked in chloroform. The powder will be percolated with at least 31 ml of chloroform at the rate of 1 drop per second. The miniature percolator will be made up of glass tubing of 8 mm bore having an over all length of 17.5 cm. One end of the tube will be sharply narrowed for about 3 cm and the other will be open out to form a flange.

To the percolate, 40 ml of 6% acetic acid in double distilled water will be added and the mixture shaken gently for 15-20 seconds. When the two layers separate out, 5 ml of the upper layer will be pipetted off and filtered through dry paper. A 1 ml aliquot of this filterate will be transferred to an evaporating dish of 5 cm diameter. The dish will be placed on water bath and the liquid will be evaporated to dryness. To the residue, 0.2 ml of fuming nitric acid (Analar) of sp. gr. 1.5 will be immediately added so as to cover the entire sample. It will then be evaporated off on the water bath in about 3 minutes. The remaining matter will be dissolved in 3 ml of dry acetone. The solution will be transferred to a 10 ml graduated test tube and the volume will be made up with the solvent. After

cooling, 0.1 ml of freshly prepared 3% solution of potassium hydroxide in methanol will be added and exactly after 5 minutes, the density of the colour observed with the help of "Spectronic 20" colorimeter at 525 nm (Peach and Tracey, 1955) and will be compared with a standard curve prepared with known amount of pure hyoscyamine sulphate in chloroform.

3.13. Estimation of chlorophyll

Chlorophyll will be estimated by the method of Arnon (1949).

1 g leaf will be taken in a mortar and grind with 40 c.c. of 80% acetone by means of pestle. It will be filtered through a Whatman No. 1 filter. The remaining leaf material will again be grind with 30 ml of 80% acetone. The process will be repeated with 20 ml and 10 ml of 80% acetone. The volume will be made upto 100 ml with 80% acetone. Per cent transmittance will be noted at various wave lengths required i.e., 663, 645 and 652 nm of "Spectronic 20" colorimeter.

Formula used

mg chl.a - per gram of tissue

$$= (12.7\ (D\ 663) - 2.69\ (D\ 645)) \times \frac{V}{1,000} \times W$$

mg chl. b. per gram of tissue

$$= (22.9\ (D\ 645) - 4.68\ (D\ 663) \times \frac{V}{1,000} \times W$$

Total Chl.

$$= \frac{D\ 652 \times 1,000}{34.5} \times \frac{V}{1.000} \times W$$

3.14 Leaf area

The area of the leaves will be measured by placing transparent graph plate on the leaf and measuring the area in sq cm. 5 leaves will be taken randomly from one pot for this purpose.

3.15 Statistical analysis

The data of all the experiments will be statistically analysed according to the design of each experiment, before drawing any conclusions.

B I B L I O G R A P H Y

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A P P E N D I X

APPENDIX

PREPARATION OF REAGENTS

Reagents for various biochemical determinations will be prepared according to the following methods.

1- Reagents for NPK

(a) Nessler's reagent

3.5 g of potassium iodide will be dissolved in 100 cc of distilled water and 4% mercuric chloride solution will be added with stirring until a slight red precipitate remains (about 325 cc of this will be required). Thereafter, 250 cc of 48% sodium hydroxide solution will be mixed to the solution and volume will be made upto 1 litre with distilled water. A little more of mercuric chloride solution will be added until a permanent turbidity develops. The mixture will be decantated and kept in brown bottle.

(b) Molybdic reagent (2.5%)

6.25 g of ammonium molybdate will be idssolved in 175 cc of distilled water to which 75 cc of 10N-sulphuric acid will be added.

(c) Aminonaphthol sulphonic acid

0.5 g of 1-amino-2-naphthol-4-sulphonic acid will be dissolved in 195 cc of 15% solution of sodium bisulphite to which 5 cc of 20% of [redacted] sodium sulphite solution will be added. The solution will be kept in brown bottle at cool place.

2- Reagent for nitrate reductase activity (NRA)

(a) Phosphate buffer (0.1 M)

13.6 g of potassium dihydrogen orthophosphate will be dissolved in 1,000 cc of distilled water (A). 17.42 g of dipotassium monohydrogen orthophosphate will be dissolved in 1,000 cc of distilled water (B) 160 cc of (A) and 840 cc of (B) will be mixed for the preparation of buffer.

(b) Potassium nitrate solution (0.2 M)

2.02 g of potassium nitrate will be dissolved in 100 cc of distilled water.

(c) Isopropanol solution (5%)

5 cc of isopropanol will be dissolved in 95 cc of distilled water.

(d) Chloramphenicol solution (0.5 mg/cc)

50 mg of chloramphenicol powder will be dissolved in 100 cc of distilled water.

(e) Sulphanilamide solution (1%)

1 g of sulphanilamide powder will be dissolved in 100 cc of 3N HCl.

(f) NED-HCl solution (0.02%)

20 mg of N-1 Nephthyl-ethylene diamine dihydrochloric acid will be dissolved in 100 cc of distilled water.

3- Reagents for Alkaloids

(a) Ethanol (95%)

95 cc of absolute alcohol will be mixed with 5 cc of distilled water.

(b) Ammonium hydroxide or Ammonia solution (10%)

10 cc of ammonia will be dissolved in 90 cc of distilled water.

(c) Acetic acid (6%)

6 cc of acetic acid will be dissolved in 94 cc of distilled water.

(d) Potassium hydroxide (3%)

3g of potassium hydroxide will be dissolved in 100 ml of methanol.